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Ex-situ cultivation at lower altitude and evaluation of *Swertia chirayita*, a critically endangered medicinal plant of Sikkim Himalayan region, India

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ABSTRACT

Swertia chirayita (family Gentianaceae) is internationally renounced and one among the 32 prioritized medicinal plants by National Medicinal Plants Board, New Delhi, Government of India. *S. chirayita* is a critically endangered plant of temperate Himalayas. Deplete population of *S. chirayita* from wild has necessitated its conservation through *ex-situ* cultivation. The study is therefore taken up for the *ex-situ* cultivation of *S. chirayita* in lower altitude (tropical hills) of Sikkim Himalayan region. The produce of the plants cultivated *ex-situ* and in niche environment was compared and evaluated as per Ayurvedic Pharmacopoeia of India (API) norms and for swertiamarin content. As per API norms the total ash and acid insoluble ash was below the permissible limits of 6% and 1%, respectively. Leaves harvested from the *ex-situ* cultivated plant showed lower 0.68% acid insoluble ash compared to 0.78% in leaves of mature plants harvested from niche environment. The alcohol-soluble extractive, water soluble extractive and total bitter content were higher (16.84%, 22.44%, and 4.57%, respectively) in leaves of the *ex-situ* cultivated plant compared to the leaves harvested from plants cultivated in niche environment (15.28%, 15.96%, and 3.95%, respectively). Besides the API norms, swertiamarin content was higher (0.27%) in leaves of the *ex-situ* cultivated plant compared to leaves harvested from plants cultivated in niche environment (0.22%). The stem harvested from plants cultivated in niche environment failed as per API specifications due to lower alcohol and water soluble extractives and very low swertiamarin content. The study shows successful harvesting of leaves with high swertiamarin content up to one year of the growth period from the *ex-situ* cultivated plants at lower altitude. Two additional harvestings are suggested from the plant cultivated in niche environment while maintaining the plant for further growth before final yield. Seeds harvested from mature plants cultivated at higher altitude are suggested for utilization for the *ex-situ* cultivation of crop at lower altitude in Sikkim Himalayan region.

1. Introduction

Swertia chirayita (Roxb. ex Fleming) Karsten (Family: Gentianaceae) is a native of temperate Himalayas growing at an altitude of 1200–3000 m above mean sea level (msl) from state of Kashmir in India to Bhutan and also in the Khasi hills of Meghalaya state at 1200–1500 m above msl (Clarke, 1885; Kirtikar and Basu, 1975; Blatter, 1984; Tandon et al., 2010). *S. chirayita* has drawn attention since ancient time due to its medicinal importance owing to the presence of bitter glycosides, xanthones, triterpenoids, iridoid and flavonoids (Negi et al., 2011; Kumar and Staden, 2016). It is one among the 32 high prioritized medicinal plants by National Medicinal Plants Board (Kala and Sajwan, 2007). *S. chirayita* is an official drug of the Indian Pharmacopoeia List 1946 and is one of the most reputed plants of Indian system of medicine (Ayurveda) (Handa, 1952; API, 2011). Significant biological activities of *S. chirayita* include anticarcinogenic, hepatoprotective, hypoglycemic, antihepatotoxic, anti-inflammatory, antimicrobial, antileprosy, antimalarial, antioxidant and CNS depressant (Negi et al., 2011). In Sikkim Himalayan region the plant of *S. chirayita* is traditionally used as home remedy for ailments such as malarial fever, indigestion, intestinal worms in children, liver diseases, cough, cold, asthma, headache, boils and scabies (Joshi and Joshi, 2008; Pradhan and Badola, 2008, 2012). *S. chirayita* is described as annual (Kirtikar and Basu, 1975; Anonymous, 1976; Bentley and Trimen, 1983; Chauhan, 1999) or a biennial (Garg, 1987; Shreshtha and Joshi, 1992; Grierson and Long, 1999; Nautiyal and Nautiyal, 2004; Joshi and Dhawan, 2005; Rijal, 2009) or triennial (Pradhan and Badola, 2012) or pluriannual *i.e.* flowering once in the third year (Edwards, 1993; Shah, 2008; Raina et al., 2013). In the first and second year of growth, the

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plant of *S. chirayita* remain in rosette form and in the later part of the second year to the third year, plant rises up (erect stage) to bear flower and fruits. Commercially the plants of *S. chirayita* are harvested during the erect stage. Farmers hesitate to take up its cultivation due to its slow growing nature (Basnet, 2001), long gestation/cultivation period of three years (Badola and Pal, 2002; Abrol et al., 2012), poor germination of seed and difficult to handle seedlings because of its very small size, *etc.* (Raina et al., 1994; Joshi and Dhawan, 2005; Chaudhuri et al., 2007). On the other hand high pharmaceutical demand, unsustainable & over harvesting from wild are causing depletion of the natural population of *S. chirayita*. Rai et al. (2000) mentioned that *S. chirayita* plants collected from Sikkim Himalaya are very high, amounting to 3,000,000 plants per year. *S. chirayita* is categorized "critically endangered" by the International Union for Conservation of Nature (IUCN) (Joshi and Dhawan, 2005). Depleting population of *S. chirayita* from wild had made necessary to take up its *ex-situ* cultivation, for conservation and to fetch the growing demand of Pharmaceutical industries (Badola and Pal, 2002; Joshi and Joshi, 2008; Pradhan and Badola, 2010a, 2010b, 2011). Badola and Pradhan (2011) mentioned *ex-situ* cultivation as an appropriate tool for *in-situ* conservation of endangered species. However, it is often opined that plant grown out of their niche environment loses their medicinal property. Understanding performance for growth, adaptability, and the metabolite of *S. chirayita* at *ex-situ* conditions of lower altitude are important. In the present study, *S. chirayita* plant cultivated *ex-situ* was evaluated for metabolite content and as per Ayurvedic Pharmacopoeia of India (API) norms. The *S. chirayita* plants were raised out of niche environment in the nursery conditions of lower altitude 800 ± 10 m above msl. *Ex-situ* produced *S. chirayita* was comparatively evaluated with the fully mature crop cultivated in the niche environment.

2. Material and methods

2.1. Ex-situ cultivation of plants

Seeds of *S. chirayita* from the niche environment collected during November 2013 were sown in the sloppy raised bed of 1 × 20 m dimension under nursery conditions at 800 ± 10 m above msl during March 2014. Depending on altitudinal elevation the area of nursery falls under tropical lower hills where the annual maximum temperature reached up to 33.51 °C (May) and minimum temperature up to 7.37 °C (January); average maximum and minimum relative humidity varied from 89.29% (June) to 39.23% (March). 1 g seed mixed with 100 g sieved and sterile wood ash spread in 1 m^2 area of sowing bed. The sowing bed mulched for one month using a double layer of shade net for a period of one month and removed thereafter. The seed started germinating after 30–35 days of sowing in the nursery bed. An emergence of 13 to 15 seedlings per 100 cm^2 area of seed bed up to 14th week of sowing and, thereafter a consistent growth in the seedlings was observed. Partial shade to germinating seedlings from a height of about 1 m by erecting a shade net with 70% cut off of sunlight was provided. Manual weeding and routine irrigation were provided to growing seedling as and when required. Routine thinning of seedlings was done to avoid internal competition. The seedling density of 40–45 plants with an average of 43 plants per square meter up to one year of the growth period was maintained. For the repetition, the field trials were again raised in 2015 with the seeds collected during November 2014. Composite data from field trials of two years are presented as the result. After a growth period of one year, leaves were harvested for yield parameters in June 2015 from 1 × 1 m^2 area of bed in eight replicates. For harvesting the leaf sample, only fully mature leaves were harvested leaving the upper 2–6 pairs of tender and newly formed leaves so that further growth of the plant can take place. Freshly harvested leaves, dried in an oven at 35 °C for 96 h were stored in the airtight container for further analysis as per API norms.

2.2. Procurement of plant sample from niche environment

For comparing the growth and yield parameters of the *ex-situ* cultivated one-year-old plant with the mature crop, the matured plants were harvested from farmer's field located in the niche environment at an altitude of 2200 m above msl. Plants were harvested from randomly scattered one square meter area in eight replicates. A number of plants per square meter ranged from 3 to 6 with an average of 4.8 plants per square meter. Root, stem and leaf parts from individual plots were oven dried at 35 °C for 96 h and dry weight was taken. Dried stem and leaf of mature plants were stored in the airtight container for further analysis as per API norms. A voucher specimen of the plant had been authenticated by and deposited at Botanical Survey of India, Sikkim Himalayan Regional Centre, Gangtok, Sikkim, India.

2.3. Parameters studied

Parameters studied under API norms included: macroscopic analysis, foreign matter (%w/w), total ash (%w/w), acid insoluble ash (%w/w), alcohol (60%v/v), soluble extractive (%w/w), water-soluble extractive (%w/w), total bitter content (%w/w) and Swertiamarin content (%w/w). Analysis of the samples as per API norms performed by the Analytical Department of Research & Development health care division of Emami Limited, Kolkata, India. Emami Limited is a pharmaceutical company under The Companies Act, 1956 and having its registered office at Kolkata, India.

2.3.1. Total ash value

For total ash value, 2 to 3 samples taken in a pre-weighed crucible and ignited at around 450 °C for 4 h in a muffle furnace. After that, the crucible was cooled down in a desiccator to room temperature and weighed. The ash value was calculated with reference to the sample weight taken.

2.3.2. Acid insoluble ash value

The ash obtained from the sample, was taken into a beaker and 25 ml dilute HCl (10% v/v) was added to it. The solution was boiled for 5 min and cooled down to room temperature. The solution obtained was filtered through Whatman No. 1 filter paper. The residue was washed with 50 ml boiling distilled water and the filter paper along with the residue then transferred into the crucible and ignited for 4 h at around 450 °C. The crucible then cooled down into a desiccator and weighed. The acid insoluble ash value was calculated with reference to the sample taken.

2.3.3. Alcohol soluble extractive value

5 g coarsely powdered sample with 100 ml of alcohol (95% v/v) was shaken frequently during the first 6 h and kept for 18 h. The sample was filtered using normal filter paper and volume made up to 100 ml using alcohol (95% v/v). From the filtrate 25 ml solution evaporated in a dry tarred Petri dish and dried to constant weight at 105 °C in hot air oven. The alcohol soluble extractive value was calculated with respect to the sample weight taken previously.

2.3.4. Water soluble extractive value

The process is same as alcohol soluble extractive value, only chloroform-water (0.25% v/v chloroform in water) was used.

2.3.5. Total bitter content

Around 3 g sample was taken and boiled in 50 ml methanol in a reflux condenser for 1 h. The solution was filtered and the filtrate was kept aside, while the residue was treated again in the same way with 50 ml of methanol. The two filtrates combined and evaporated completely in a water bath. 30 ml of water was added to the residue obtained after evaporation and sonicated for 10 min to dissolve. The solution was filtered using normal filter paper and the filtrate was taken into a

250 ml separating funnel. 3 × 25 ml of ethyl acetate was added and shaken for 1 min each time. Ethyl acetate layers separated each time was combined together while the aqueous layer discarded. The ethyl acetate solution evaporated on tarred Petri dish and dried to constant weight at 105 °C in hot air oven. The Petri dish was weighed and bitter content was calculated with reference to the sample taken.

2.3.6. Quantitative estimation of Swertiamarin

The plant material cleaned and dried in the shade for a week at room temperature was powdered to 40 meshes and stored at 25 °C. Standard compound swertiamarin was purchased from Natural Remedies Pvt. Ltd. (Bangalore, Karnataka, India). All the solvents and reagents used in the experiments were of analytical grade.

2.3.6.1. Preparation of mobile phase & standard solutions. The mobile phase was prepared by mixing Ethyl acetate: Methanol (8:2). A stock solution of swertiamarin (5 mg/ml) was prepared by dissolving 50.0 mg of the standard swertiamarin accurately weighed in 10 ml methanol in a volumetric flask. Appropriate quantities of this standard solution were spotted to obtain swertiamarin.

2.3.6.2. Preparation of sample solutions. Powdered samples of Chirata aerial parts (5 g, accurately weighed) were extracted with methanol (2 × 25 ml) by sonicating for 5 min and heating on a water bath for 10 min. The solutions were filtered using Whatman No. 1 filter paper. The filtrates combined and volume was reduced to 10 ml by low-temperature evaporation.

2.3.6.3. Instrument and chromatographic conditions. HPTLC was performed on 20 × 10 cm aluminum backed plate coated with 0.2 mm thick layer of silica gel 60 F254 (E-Merck, Germany). Standard solutions of swertiamarin and the samples applied to the plates as 6 mm wide band, 8.9 mm apart and 8 mm from the bottom and sides using a CAMAG Linomat V sample applicator were attached to a CAMAG Microlitre syringe (CAMAG, Germany). Linear ascending development of the plates up to an 80 mm distance was performed with Ethyl acetate: Methanol (8:2), as mobile phase in a 20 × 20 twin trough glass chamber was previously saturated with the vapor of mobile phase for 15 min at 25 ± 2 °C. The plate dried completely and was scanned at 245 nm by a CAMAG TLC scanner in absorbance mode, using the deuterium lamp.

3. Results

Data from Table 1 reveal that per square meter (sqm) dry weight yield of the root, stem and leaves were 10.27 g, 84.86 g, and 33.78 g, respectively in fully mature plant harvested from niche environment (Fig. 1A, B). The leaf yield from the *ex-situ* cultivated one-year-old plant was 28.15 g per sq. m. No root was harvested from *ex-situ* cultivated plant so as to maintain the plant for further growth; similarly stem also not harvested as its formation takes place after second year onwards (Fig. 1C, D). The projected per hectare yield of dry stem and leaves from fully mature plant harvested from niche environment was around 849 kg and 338 kg, respectively; whereas leaf yield from one-year-old *ex-situ* cultivated plant at lower altitude was around 280 kg per hectare (Fig. 2). Macroscopic analysis of plant material showed that all samples comply with the API norms. The whole dried plant material is used in the form of the drug. The harvested plant material consisted of the stem up to 1 m long and 6 mm in diameter, glabrous, yellowish-brown to purple, feebly quadrangular above and cylindrical below with large, continuous and easily separable yellow pith. Leaf characteristics include opposite, cauline, broad at base, ovate or lanceolate, entire, acuminate and, glabrous. However, leaves of one-year-old *ex-situ* cultivated plant were radical forming in rosette, simple, sessile, oblanceolate to spathulate, acute or obtuse and larger in size as compared to the leaf of the erect stage of a fully mature plant (Fig. 1E). Total ash and acid insoluble ash ranged from 2.86% to 5.55% and 0.39% to 0.78%, respectively in plant samples. Minimum percentage of total ash and acid insoluble ash was in the stem of fully mature plant cultivated in the niche environment. Maximum percentage of total ash and acid insoluble ash was in leaf sample of one-year-old *ex-situ* cultivated plant and in the leaf of fully mature plant cultivated in niche environment, respectively. Alcohol and water soluble extractives ranged from 6.27% to 16.84% and 7.82% to 22.44%, respectively in plant samples. The stem of fully matured plant cultivated in niche environment showed the lowest percentage of alcohol and water soluble extractives. Maximum percentage of both alcohol and water soluble extractives was observed in the leaf of the one-year-old *ex-situ* cultivated plant. Percentage of total bitter content was the highest 4.57% in the leaf of the one-year-old *ex-situ* cultivated plant at lower altitude, followed by 3.95% in leaf and lowest 2.15% in the stem of fully mature plant cultivated in the niche environment. Similarly, swertiamarin content was the highest 0.27% in the leaf of one-year-old *ex-situ* cultivated plant at lower altitude, followed by 0.22% in leaf and lowest 0.002% in the stem of fully mature plant cultivated in the niche environment (Fig. 2; Table 2). Dark zones with well defined peaks observed at R_f value of 0.40 for standard swertiamarin (Fig. 3a, b). Samples of fully mature niche environment cultivated stem showed very light zone as compared to leaf with R_f value of 0.41 and 0.34, respectively (Fig. 3a, c, d). Whereas, leaf of the one year old *ex-situ* cultivated plant showed dark zone for swertiamarin presence with R_f value of 0.42 at 254 nm. (Fig. 3e)

4. Discussion

The necessity of conservation of high priority, endangered medicinal plant *S. chirayita* through *ex-situ* cultivation and development of agro-techniques had been optioned by various workers (Joshi and Dhawan, 2005; Joshi, 2008; Joshi and Joshi, 2008; Phoboo and Jha, 2010; Pradhan and Badola, 2010a, 2010b; Badola and Pradhan, 2011; Tabassum et al., 2012; Purohit et al., 2013). The long gestation period of crop discourages the farmers to take up its commercial cultivation. However, *S. chirayita* has high pharmaceutical importance. The plant demand is fetched up by sporadic cultivation by farmers, along with the collection from wild for local needs. The uncertainty of the market and fluctuating market rate of produce also tends to discourage the cultivation of *S. chirayita* and therefore tend to increase the practice of collection from wild at the time of the sudden rise in market demand of the plant. Badola and Pradhan (2011) mentioned non-availability of quality planting material, poor development & extension support in the cultivation & processing and unorganized markets, *etc.* are major constraints hindering the commercial cultivation of *S. chirayita*. At present Nepal is the major exporter of *S. chirayita* exporting more than 45% of the world total volume (Rai et al., 2000; Barakoti, 2004; Joshi and Dhawan, 2005; Phoboo and Jha, 2010; Niroula and Kumari, 2012; Khanal et al., 2014). Export of *S. chirayita* from Nepal was 419 ton in 1995–96 followed by 82.8 metric ton in 1998–99 and 82.2 metric ton in 2004–05 (GoN, 1998–2005; Niroula and Kumari, 2012). The demand

Table 1
Dry biomass of *Swertia chirayita* mature plant from niche environment and one-year-old *ex-situ* cultivated plant at the lower altitude.

S. no.	Growth stages	Yield (g per sq. m)		
		Root	Stem	Leaves
1	Mature plant from niche environment (3–6 plants with average of 4.8 plants per square meter)	10.27 ± 5.53	84.86 ± 28.26	33.78 ± 10.77
2	One-year-old plant cultivated at out of niche environment (40–45 plants with average of 43 plants per square meter)	Not harvested	Not formed	28.15 ± 6.59

Fig. 1. Fully mature crop cultivated in niche environment (a), fully mature dried harvested plants from niche environment (b), one-year-old *ex-situ* cultivated plants at lower altitude (c), freshly harvested leaves from one-year-old *ex-situ* cultivated plants (d) and leaf: sessile leaves of fully mature plant cultivated in niche environment (left) and petiolated leaves of one-year-old *ex-situ* cultivated plants (right) (e).

of *S. chirayita* is gradually increasing in domestic and pharmaceutical industries within and outside India. An estimated annual trade of *S. chirayita* in 2005–2006 was 500–1000 MT (Samaddar et al., 2014) whereas; official import was 97.4 MT (Ved and Goraya, 2007). Sikkim state of Indian is adjoining to Nepal and harbors a very congenial environment for the commercial cultivation of *S. chirayita*. However, *ex-situ* cultivation of *S. chirayita* especially at lower altitudes of Sikkim has not been tried. In the present study, the *ex-situ* cultivated plants at lower altitude yielded 28.15 g dry leaf per sq. m in one year, whereas total biomass of mature plants cultivated in niche environment was 128.91 g per sq. m. The projected per hectare dry leaf yield of the one-year-old *ex-situ* cultivated plant at lower altitude was 280 kg, which is less than the per hectare dry leaf yield of 338 kg per hectare from the mature plant (Fig. 2). But, the noteworthy thing is that to reach the maturity period plant takes two plus year's time, during which the *ex-situ* cultivated plant, can yield twice. Badola and Pradhan (2011) carried out cultivation of *S. chirayita* in the altitude of occupancy at 2000 m above msl, observed maximum (42.6 g) dry biomass per plant in an open bed, 37.79 g in the greenhouse and 31.52 g in poly house conditions. They advocated open bed condition as much feasible and profitable for large-scale cultivation of *S. chirayita*. In the present study, the one-year-old *ex-situ* cultivated plant at lower altitude had shown a better alternative rather than to wait for two plus years for yield as in the conventional method of cultivation. In the analysis of herbal samples as per API norms, the total ash reflect the plant tissue derived physiological ash and acid insoluble ash depicts the non-physiological ash from environmental contaminants such as sand and soil. Both total ash and acid insoluble ash contents are indices of quality and purity of herbal medicine (Rao and Xiang, 2009). In the present study, the total ash in the leaf sample of *ex-situ* cultivated plants at the lower altitude was high 5.55% as compared to the 5.37% in leaves of mature plant cultivated in niche environment, but both were below the permissible limit of 6.0% as per required API specification. Sayyed et al. (2014) observed total ash 4.9%

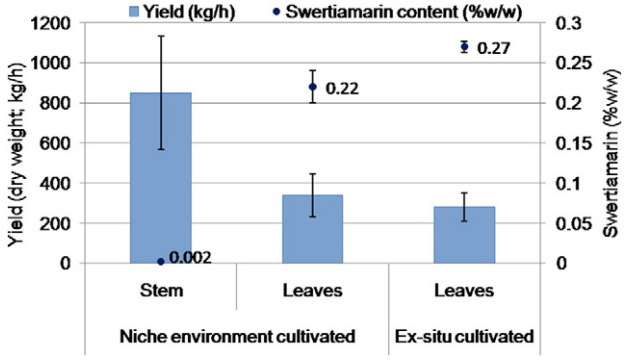

Fig. 2. Projected yield of *Swertia chirayita* mature plant from niche environment and one-year-old *ex-situ* cultivated plant at the lower altitude with their respective swertiamarin content.

Table 2
Analysis of *Swertia chirayita* mature plant cultivated at an altitude of niche environment (niche environment cultivated) and one-year-old *ex-situ* cultivated plant at the lower altitude (*ex-situ* cultivated) as per Ayurvedic Pharmacopoeia of India (API) norms.

S. no.	Parameters	API specifications	Stem of niche environment cultivated	Leaves	
				Niche environment cultivated	*Ex-situ* cultivated
1	Macroscopic description	The drug consists of whole plant, glabrous, yellowish-brown stem with easily separable yellow pith, leaf, ovate or lanceolate, entire, acuminate, glabrous.	Stem up to 1 m long and 6 mm in diameter, glabrous, yellowish-brown to purplish, slightly quadrangular above and cylindrical below, large, continuous, easily separable yellow pith.	Leaf, opposite, cauline, broad at base, ovate or lanceolate, entire, acuminate, glabrous.	
2	Foreign matter (%w/w)	Not >2.0	Nil	Nil	Nil
3	Total ash (% w/w)	Not >6.0	2.86 ± 0.34	5.37 ± 0.14	5.55 ± 0.07
4	Acid insoluble ash (%w/w)	Not >1.0	0.39 ± 0.13	0.78 ± 0.03	0.68 ± 0.05
5	Alcohol (60% v/v) soluble extractive (%w/w)	Not <10.0	6.27 ± 0.62	15.28 ± 3.29	16.84 ± 0.08
6	Water soluble extractive (% w/w)	Not <10.0	7.82 ± 1.34	15.96 ± 1.45	22.44 ± 0.40
7	Total bitter content (%w/w)	Not <1.30	2.15 ± 0.42	3.95 ± 0.17	4.57 ± 0.00
8	Swertiamarin content (% w/w)	–	0.002 ± 0.00	0.22 ± 0.02	0.27 ± 0.007

and acid insoluble ash 0.96% in *S. chirayita*. The 0.68% and 0.78% acid insoluble ash value in leaves of *ex-situ* cultivated plants at the lower altitude and mature plant cultivated in niche environment, respectively, showed that environmental contaminant was less as compared to the observations made by Sayyed et al. (2014). The stem of mature plant cultivated in niche environment had shown comparatively less total ash value 2.86% and acid insoluble ash value of 0.39%. The alcohol soluble and water soluble extractive values provide information about the active constituents of the herbal material. These extractive values play an important role in establishing the standards for an herbal drug. As per API specifications both the alcohol soluble and water soluble extractive values should not be less than 10% for *S. chirayita*. In the present study both alcohol and water soluble extractive values were higher (16.84% and 22.44%, respectively) in leaf sample of one-year-old *ex-situ* cultivated plant, followed by (15.28% and 15.96%, respectively) in leaf sample of mature plant cultivated in the niche environment. The stem sample failed as per required API norms due to low alcohol soluble extractive 6.27% and water soluble extractives 7.82%. Sayyed et al. (2014) observed 12.39% alcohol soluble extractive and 13.17% water soluble extractives in *S. chirayita* collected from the market sample. The results of alcohol soluble and water soluble extractive values of leaf samples in present study had shown better results as compared to observations made by Sayyed et al. (2014) from market sample. In the present study, the swertiamarin and the total bitter content in leaves of *ex-situ* cultivated plant was high 0.27% and 4.57% as compared to the leaves of mature plant 0.22% and 3.95%, respectively. The swertiamarin and the total bitter contents in the stem of mature plant were very less (0.002% and 1.83%, respectively) as compared to leaves. Barakoti et al. (2012) observed 0.95 to 2.0% bitter principle among the tested samples from fourteen (including four cultivated and ten wild) populations of *S. chirayita* collected from eastern, central and western hill districts of Nepal. Barakoti et al. (2012) advocated that sample collected from wild population in general contain higher bitter principle, which also varies depending on agro-ecological growing conditions. Kumar and Chandra (2015) developed a rapid analytical method to determine xanthone and secoiridoid glycoside, found variation in the concentration of mangiferin, amarogentin and swertiamarin in *in-vitro* and *in-vivo S. chirayita*. Butt et al. (1999) observed variation ranging from 0.75% to 1.14% in bitter content in sixteen populations of *S. chirayita* distributed along 1500 m to 2700 m altitude of Himachal Pradesh, India. In the present study the total bitter content in stem and leaf samples is not only above the required API specification of 1.3%, but also better than the observations of earlier workers. Phoboo et al. (2010) observed swertiamarin content ranging from 1.15 to 1.28 mg/g dry weight in inflorescence and leaf mixture of plant sample collected from nine different districts of Nepal. Observations of the present study have shown the swertiamarin content better in leaf sample of both *ex-situ* cultivated plant at lower altitude and from mature plant cultivated in niche environment, than the earlier studied populations of Nepal. Commercially the produce of *S. chirayita* is sold as whole uprooted plant material and not separately as leaf and stem. The harvesting of leaves during first and second year of growth phases can provide additional income to cultivators. In the present study the leaves of the plant cultivated *ex-situ* at lower altitude and in niche environments have shown higher swertiamarin content as compared to stem. However, stem sample failed as per API specification due to low alcohol soluble and water soluble extractive values. Metabolite content and other specifications as per API norms also depend on the storage conditions, post harvest handling, geographical location of plant growth and climatic conditions during harvest, *etc.* of the produce (Khanal et al., 2014). Farmers generally dry the harvested plant material in sunlight. However, in the absence of enough sunshine farmers stack the plant material at the attic of their houses. This creates congenial conditions for fungus and bacteria growth leading to low quality product (FRDD, 2008). Inappropriate drying and post-harvest handling of the crop lead to loss of most of the leaves through shedding or decay. Farmer can suffer a loss of 30% of produce from harvesting to selling because of inappropriate drying, packaging and storage (FRDD, 2008). Biomass of plant and its metabolite content also depends on the growth stage of plant. Bhatt et al. (2006) observed no significant difference in biomass of plants harvested during flowering and senescence stage, indicating that both stages as equally good for optimum biomass harvest; however, he further emphasized for detailed study of the active compound available in the two growth stages. Kumar and Chandra (2015) observed higher concentration of mangiferin and swertiamarin in *in-vitro* regenerated plant compared to wild plant; while amarogentin concentration was high in wild plant compared to *in-vitro* regenerated plants. Phoboo et al. (2010) observed no significant difference in the amounts of amarogentin, mangiferin and swertiamarin content in wild and cultivated plant parts of *S. chirayita*. Maximum content of these phytochemical observed in mixture of inflorescence and leaf as compared to stem and root (Phoboo et al., 2010). Result of the present study shows that the leaves with high swertiamarin content can be sustainable harvested from the plants within one year of growth period. As the need for *ex-situ* cultivation of *S. chirayita* felt by many workers; the present study and its finding provide important information. Firstly, the successful cultivation of *S. chirayita* at lower altitude of 800 ± 10 m above msl. Secondly, yield of *S. chirayita* in first year when the plants are in rosette stage while maintaining the plants for further growth under nursery or field conditions. However, technique of harvesting the leaves of plant after one year suggested applying successfully for cultivation at higher altitude too. This maximizes the yield by two more harvestings during the gestation period of crop. Seeds harvested from mature plants cultivated at higher altitude are suggested for utilization for *ex-situ* cultivation of

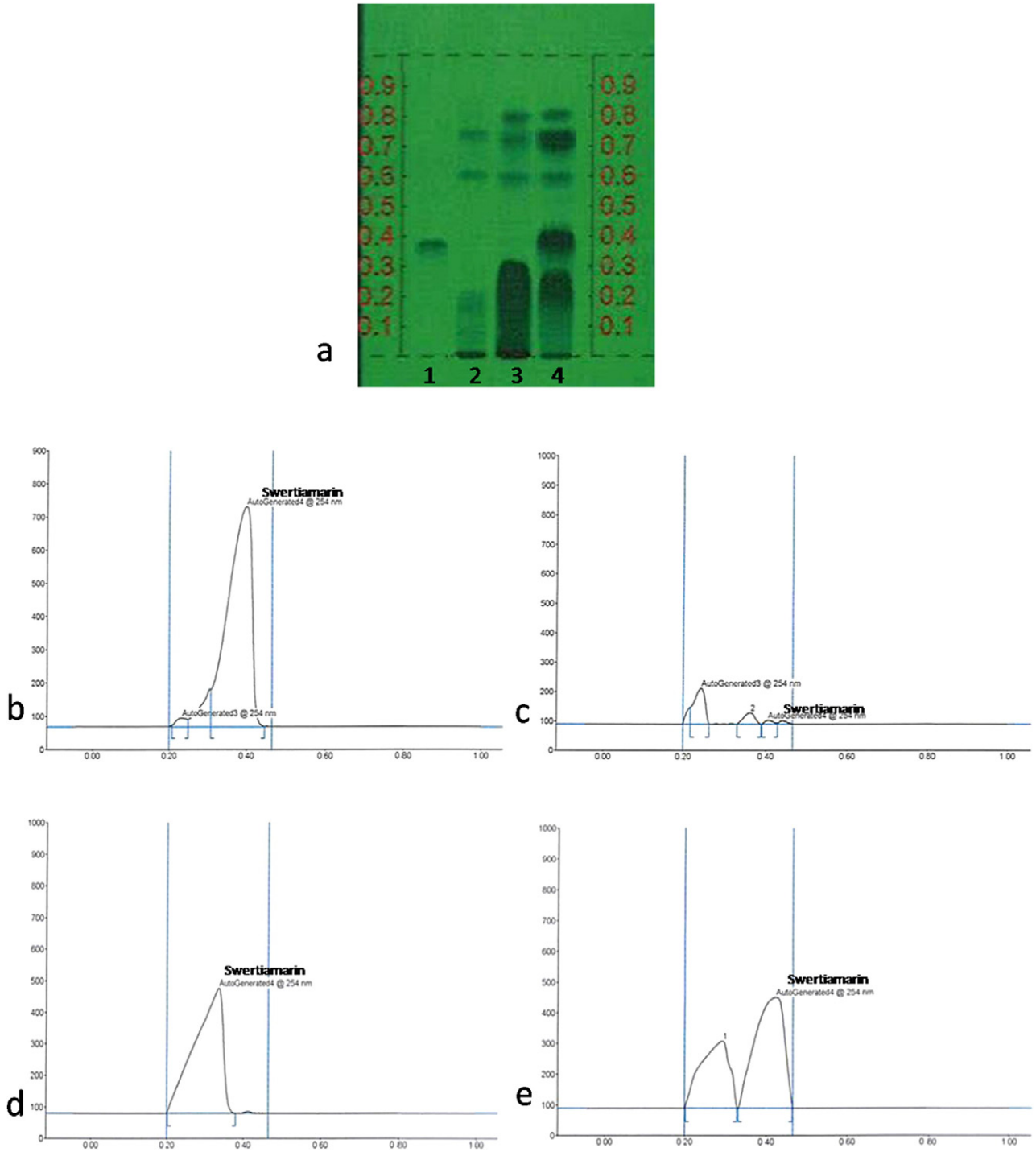

Fig. 3. An image of HPTLC plate at UV 254 nm showing swertiamarin content, track 1: standard swertiamarin, track 2: swertiamarin content in stem (niche environment), track 3: swertiamarin content in leaves (niche environment), track 4: swertiamarin content in leaves (*ex-situ* cultivated plant) (a); HPTLC densitogram of swertiamarin standard (b), stem (niche environment) (c), leaves (niche environment) (d) and leaves (*ex-situ* cultivated plant) (e), [x-axis represent retention factor (R_f) & y-axis represent absorption unit (AU)].

crop at lower altitude in Sikkim Himalayan region. This method of cultivation utilized in most of the crops, in which leaves are the main produce. This study provides a successful example of cultivation and harvesting of *S. chirayita* within one year at tropical hill below the altitude of occupancy. However within the altitude of occupancy *i.e.* 1200–3000 m above msl possibility of taking two additional yields exists before the final yield. In the present study, the *ex-situ* cultivation at the lower altitude of 800 ± 10 m above msl faced many bottlenecks. Major among them were initial germination of seed & slow seedling growth, competition with weed & subsequent loss of seedlings during weed removal. Pradhan and Badola (2011) observed the non-uniform emergence of seedlings as a big constraint in commercial cultivation of *S. chirayita*. *S. chirayita* is a slow growing plant, therefore cannot compete with simultaneously growing weed. Large grown weed when uprooted, brings out the small seedling of *S. chirayita* too. Therefore it is important to remove weed at appropriate stage so that uprooting do not disturb the growing *S. chirayita* seedlings. Another problem faced during cultivation at lower altitude was of attack of pests on plant. The sludge and sails attacked on the lower mature leaves of plant mainly during night. Snail and sludge subsequently start feeding

on young and growing leaves too. Therefore harvesting of the mature and fully grown lower leaves as a control measure of snail and sludge is advised for organic cultivation. Moisture accumulation in soil bed during monsoon is detrimental to the growth of *Swertia* plant. Badola and Pradhan (2011) recommended avoidance of irrigation, as sloppy soil condition results in root decay. The *ex-situ* cultivated plants of *S. chirayita* could successfully grow up to 14 to 15 months but, did not reach to flowering stage due to moisture and higher temperature conditions of lower altitude. For removal of water and accumulating moisture, more studies in terms of design, direction and slope of bed required through different agro-techniques, so that plant could reach the flowering and fruiting stage when cultivated at lower altitude. Bhattarai and Shreshta (1996) observed that for cultivation of *S. chirayita* north facing gentle slopes which get morning and afternoon inclined sun rays is favorable. The *ex-situ* cultivation at lower altitudes not only leads to *in-situ* conservation but also fulfills the industrial requirement of raw material. It can also overcome problem related to misidentification, contaminants and variability in active metabolite of produce (Phoboo and Jha, 2010). The *ex-situ* cultivated plant at lower altitude of 800 ± 10 m above msl showed a way forward for *in-situ* conservation of plant; however research introspections for location specific climatic conditions, soil profile & composition and microenvironment required. The adoption of methodology based on above criteria with adoption of some agro-techniques, so as to get relatively better and consistent performance of plants to reach the full maturity stage at lower altitudes may be beneficial for large scale *ex-situ* production of *S. chirayita*. These studies and their findings are important in the development of management strategies for conservation & introduction of plant into *ex-situ* cultivation at lower altitude and are especially relevant in Eastern Himalayan region of Sikkim.

5. Conclusion

The study shows the performance of *ex-situ* cultivated *S. chirayita* at an altitude of 800 ± 10 m above msl in tropical hills of Sikkim Himalayan region. Finding shows successful raising of *S. chirayita* for a period of one year with the produce standing fit as per API specifications with high swertiamarin content. The study also suggests for harvesting two additional yields during first and second years before the final yield at maturity under cultivation in the niche environment.

Conflict of interest

The authors declare that there is no conflict of interest.

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